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LIPID PROFILE OF MALARIA INFESTED WISTAR ALBINO RATS TREATED WITH THE CRUDE EXTRACT OF *ARTEMISIA ANNUA* AND ARTEMISININ COMBINATION THERAPY.

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ABSTRACT

The comparative effects of the crude extract of *Artemisia annua* and artemisinin combination therapy (ACT) on the lipid profile of wistar albino rats was evaluated in this study. Twenty four (24) rats used were divided into four (4) groups of six rats each. Group 1 (control) and 2 (malaria untreated) received 0.2ml of distilled water while group 3 (ACT treated) and 4 (*A. annua treated*) received 1.43mg/kg and 300mg/kg of ACT and *A. annua* extracts respectively. Treatment was administered twice a day for three consecutive days, after which blood sample was taken through cardiac puncture into treated sample bottles for analysis. The results showed that, TC, TG, HDL and LDL were significantly reduced (P<0.05) in both the malaria untreated and ACT treated groups when compared with the control while TC and LDL were significantly increased (P<0.05) in the *A. annua* treated group. The HDL and VLDL levels showed no significant change compared with the control. The results obtained from this study showed that, the TC levels for the experimental animals were within the normal physiological range except for the malaria untreated group which showed decreased levels, hence a lose in body weight. In this study, both ACT and the crude extract of *Artemisia annua* were compared, and it was discovered that both proved to be effective with no noticeable side effects, hence should be encouraged.

KEYWORDS: Lipid, *Artemisia annua*, *Plasmodium*, artemisinin, phytochemicals, flavonoids, serum.

INTRODUCTION

Malaria is one of the infectious diseases that have caused so much death globally. It is caused by a parasitic protozoan called *Plasmodium* which is commonly transmitted by the bite of an

infected female anopheles mosquito. Malaria can be caused by any one of the five *Plasmodial* parasites, *P. vivax, P. Ovale, P. fulciparum, P. malariae* or *P. knowlesi.* Of these, the most deadly is the *P. fulciparum,* however *Plasmodium knowlesi* rarely cause malaria in human. Malaria is usually diagnosed by the microscopic examination of blood sample using blood films or with the use of antigen-base rapid diagnostic tests (Caraballo, 2014) and by the use of a malaria test-kit such as Care Start™ malaria HRP2 test kit (Sun *et al*., 2013). The symptoms of malaria usually begins about five to eight days following a bite by an infected mosquito and these include fever, headache, dizziness, nausea, vomiting, joint pains, dry cough, etc. (fairhust *et al*., 2010).

The phytochemical screening of *Artemisia annua* revealed the presence of flavonoids, essential oils, terpenoids, coumerins, phenolics and most especially artemisinin (Oriakpono *et al.*, 2012) from which ACT are synthesized.

Lipid profile is a test used to assess and/ or determine the risks of a cardiac or heart disease. Its aim is to identify various disturbances in the cholesterol and triglycerides levels which are implicated in cardiovascular diseases. Lipids are a group of fats and fat-like substances that are important constituents of cells and which also serve as sources of energy. It is imperative to monitor and maintain an appropriate serum lipid levels for a healthy living. Lipid profile include assessing total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides (TG), very low density lipoprotein cholesterol (VLDL-C), etc. Because of the role lipid plays in various cardiovascular diseases, it became necessary to investigate the effect of the crude extract of *A. annua* and artemisinin combination therapy (ACT) on lipid profile of malaria infested wistar albino rats.

MATERIALS AND METHODS

Collection and preparation of materials

ACTs, Dextrose and Distil Water: The anti malarial drug zymal® (Artemether Tablet 80mg + Lumenfantrine Tablets 480mg) manufactured by Innova CapTab, Pharmaceutical Co Ltd, 81-B EPIP, Phase-I, Jharmajri, Baddi (H.P) India. Manufacturing Lic. No.: MNB/06/394, Distilled water and 5% dextrose water was bought from Turtle Bay pharmacy in Calabar.

Plant material: The leaves of *Artemisia annua* was collected from the biotechnology farm owned and operated by Prof. Ebiamadon Andi Brisibe, of the Department of Genetics and Biotechnology, Faculty of Science, University of Calabar. It was taken to the Botany

Department of the University for Identification and specimens were deposited at the department's herbarium.

Parasites: The strain of *Plasmodium falciparum* used for this study was obtained and authenticated by the Calabar office of Roll Back Malaria.

Laboratory animals: A total of 24 Inbreed adult male and female wistar albino rats weighing between 180 - 220g were used for this study, they were purchased from the animal house unit of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, and were housed in a well ventilated wooden cages in the animal house, and were fed with rat pellets (growers' marsh manufactured by Vital feeds Ltd, Lagos) and tap water *ad libitum.* The animals were acclimatized for three weeks and their body weights noted before and after the commencement of the experiment. The animals were divided into four groups, based on their weights as shown in table 1.

Innoculation: The infection of the recipient rats was initiated by injection of the parasites preparation authenticated by the Calabar office of Roll Back Malaria to healthy test rats via intramuscular route as described by David *et al* (2004) and Peter and Anatoli (1998). 2ml of *Plasmodium falciparum* sample with a parasite load of 161.5 was diluted with 5% dextrose water using a dilution factor of 1:4 (Shakya *et al.*, 2012). 0.5ml per kilogram of body weight of the diluted plasmodium base solution was subsequently injected into the animals in group 2, 3 and 4 via intramuscular method (David *et al.*, 2004).

Determination of Degree of Parasitaemia: The CareStart™ Malaria HRP2 Pf (Cat #: G0141) test kit, manufactured by Access Bio, Inc. 65 Clyde Road, Somerset, NJ, 08873, USA, was used to investigate the level of infection in the groups of rats that were inoculated with the malaria parasites. 48 hours after inoculation, a drop of blood was collect from the tails of the infected rats and tested for the presence of *plasmodium* according to the method describe by the manufacturer.

Administration of Drug: The antimalarial drug, zymal® (Artemether 80mg + Lumenfantrine 480mg) tablet was used as the artemisinin combination therapy. It was powdered in a mortar, mixed with 50ml distilled water and administered as aqueous suspension by oral gavage at a dose of 1.143mg/kg body weight twice a day for three consecutive days.

Administration of Extract: 40g of the powdered *Artemisia annua* leaves was socked in ethanol for 12 hours and filtered thereafter. The filtrate was further filtered using a watman filter paper and then concentrated by evaporation using a water bath at 40^{o}C. The 40g of powdered *A. annua* leaves yielded 2.9g of extract. The crude extract was administered to group 4 animals at a dose of 300mg/kg body weight twice a day for three consecutive days.

Collection and preparation of tissue for analysis: After 3 days of treatment, the rats were weighed and fasted overnight. Blood samples were collected from the untreated, treated and control groups for investigation of lipid profile. The animals were anaesthetized with trichloromethane (chloroform). They were then dissected and blood samples were collected through cardiac puncture using sterile syringes into screw cap sterile test tubes.

Assessment of lipid profile: the serum total cholesterol and triacylglycerol levels were determined using the calorimetric method a described by the manufacturers of Randox diagnotic test kits (Seidel *et al.*, 1984). While the levels of HDL, LDL, and VLDL – cholesterol concentration was determined using the calorimetric method described by Dialab diagnostic kit manufacturers (Friedwaid *et al.*, 1972).

Statistical analysis: Data was expressed as mean ± standard error of mean. The data obtained were analyzed statistically using one-way analysis of variance (ANOVA) at a 95% (0.05) probability level.

RESULTS

Table 2 shows the results of lipid profile, TC (mg/dl), TG (mg/dl), HDL (mg/dl), LDL (mg/dl), VLDL (mg/dl) and $\frac{LDL}{HDL}$.

Effect of treatments on total cholesterol (TC) level: the results as presented in Table 2 showed that the total cholesterol level in malaria untreated group (1.21±0.08) and ACT treated group (1.57±0.18) were significantly lower ($P<0.05$) when compared with the control (1.57±0.20). While that of *Artemisia annua* treated group (1.72±0.12) was significantly higher ($P<0.05$) compared with the control. More so, the *Artemisia annua* treated group is significantly higher than both the ACT treated and malaria treated groups ($P<0.05$).

Effect of treatment on triacylglycerol (TG) level: the results also reveals that the serum level of triacylglycerol for malaria untreated group (0.82±0.16), ACT treated group

(0.77±0.07) and *Artemisia annua* treated group (0.82±0.21) were all significantly lower (P<0.05) when compared with the control (1.05±0.28). TG result for the *A. annua* treated group is non-significantly higher than the ACT treated group.

Effect of treatment on high density lipoprotein (HDL) level: the result of the research also showed that the serum level of HDL for the malaria untreated group (0.31±0.02) and ACT treated group (0.33±0.04) were both significantly lower (P<0.05) compared with the control (0.40±0.05) while that of the *A. annua* treated group (0.43±0.03) showed no significant change compared with the control at P>0.05. The *A. annua* treated group is also significantly higher than both the malaria untreated and ACT treated groups at P<0.05.

Effect of treatment on low density lipoprotein (LDL) level: the result of the serum level of LDL obtained showed that the LDL level of the malaria untreated group (0.54±0.06) and ACT treated group (0.56±0.15) were significantly lower when compared to the control (0.70±0.08) while that of *A. annua* treated group (0.89±0.14) is significantly highly when compared to the control at P<0.05. More so, the serum LDL level of the *A, annua* treated group is significantly higher than both the malaria untreated and ACT treated groups at P<0.05.

Effect of treated on very low density lipoprotein (VLDL) level: the result obtained also showed that the VLDL level of both the malaria untreated group (0.37±0.07), ACT treated group (0.36±0.03) and *A. annua* treated group (0.37±0.09) showed no significant change (P<0.05) compared with the control. There is also no significant change among the three groups at P<0.05. The $\frac{LDL}{HDL}$ ratio of *A. annua* treated group is significantly higher (P<0.05) than both the control, malaria untreated and ACT treated groups while that of the other groups showed no significant changes compared with the control at P>0.05.

Table 1: Experimental protocol

Experimental group distribution of wistar albino rats during treatment with anti malarial drug (ACT) and extract from *Artemisia annua* leaves.

Groups	Numbers of rats	Treatments
1	6	Normal control
2	6	Malaria infected and untreated
3	6	Malaria infected and ACT treated
4	6	Malaria infected and *Artemisia annua* treated

Table 2: Lipid profile baseline values

Effects of Artemisinin Combination Therapy (ACT) and *A. annua* extract on lipid profile of malaria infested wistar albino rats.

Treatments	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	$\frac{LDL}{HDL}$
Control	1.57±0.20	1.05±0.28	0.40±0.05	0.70±0.08	0.48±0.13	1.75
Malaria untreated	1.21±0.08[a]	0.81±0.16[a]	0.31±0.02[a]	0.54±0.06[a]	0.37±0.07	1.74
ACT treated	1.24±0.18[a]	0.77±0.07[a]	0.33±0.03[a]	0.56±0.15[a]	0.36±0.03	1.70
A. annua Treated	1.72±0.12[a,b,c]	0.82±0.21[a]	0.43±0.03[a,b,c]	0.89±0,14[a,b,c]	0.37±0.09	2.07[a,b,c]

Values expressed as Mean±SEM, n=6, P<0.05

a significantly different from control (group 1) at P<0.05

b significantly different from group 2 at P<0.05.

c significantly different from group 3 at P<0.05.

DISCUSSION

The result obtained for lipid profile showed that the TC level for the experimental animals were within the normal physiological range except for the malaria untreated group that showed a decreased TC level, hence showed a remarkable lose of body weight. Generally, there was a marked decrease in the serum level of TG and VLDL and an increase in the LDL and TC levels of group 4. From the result, the extract of *A. annua* with significantly higher levels of TG and LDL may impose more threat to health than the ACTs, since these lipid fractions are implicated in the development of cardiovascular diseases (Vasudevan and Sreekuman, 2007).

CONCLUSION

In this study, it has been revealed that the crude extract of *Artemisia annua* proves as useful as the refined ACT. Therefore, where the ACTs are not readily accessible, monitored and regulated use of the extracts should be encouraged.

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